BIOGRAPHICAL SKETCH

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NAME: Daniela Nicastro

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Associate Professor of Cell Biology and Biophysics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Ludwig-Maximilians University, Munich, Germany	Dipl. Biol.	1995	Biology
Ludwig-Maximilians University/ Max-Planck-Institute for Biochemistry, Munich, Germany	Ph.D.	2000	Biology
Max-Planck-Institute for Biochemistry, Munich, Germany	Postdoc	2000-2001	Structural Biology
University of Colorado at Boulder, CO	Postdoc	2001-2006	Struct./Cell Biology

A. Personal Statement

Fascinated by the complexity and function of cells, I'm drawn to important, yet challenging cell biological and biomedical questions. I have more than 20 years of experience in electron microscopy of cellular structures and I'm a leading expert in cellular cryo-electron tomography, but my general approach to science is to pursue exciting questions and ideas by whatever (interdisciplinary) means necessary. The research interest of my laboratory focuses on the three-dimensional structures and functions of organelles and macromolecular complexes, with special emphasis on cytoskeletal assemblies and molecular motors. We use a combination of cutting-edge methods, including *in situ* molecular imaging using cryo-electron tomography combined with sub-tomographic averaging, correlative light and electron microscopy, biochemical and mass spectrometric analyses, integrated structural-genetics approaches and protein labeling techniques to directly visualize gene products in cells in their living state. We aim to understand how proteins interact, work, and are spatially arranged within cells, which should ultimately enhance our understanding of fundamental processes underlying cellular functions that are integral to the health of all living organisms. Our most recent data provide new insights into the structure of the molecular motor dynein and the structural proteome of cilia and flagella, which are organelles with important biological roles in cell motility and sensation.

B. Positions and Honors

Research and Employment

2000-2001 Postdoctoral research with Dr. Wolfgang Baumeister, MPI for Biochemistry, Munich, Germany
2001-2006 Postdoctoral research with Dr. Richard J. McIntosh, Univ. of Colorado, Boulder, CO
2006-2013 Assistant Professor of Biology, Brandeis University, MA
2013-2015 Associate Professor of Biology, Brandeis University, MA
2015 July - Associate Professor of Cell Biology and Biophysics, UT Southwestern Medical Center, TX

Other Experience and Professional Memberships

Editorial Board member: Cytoskeleton
Reviewer of grant proposals: National Science Foundation.

Reviewer for journals: Journal of Structural Biology, Journal of Molecular Biology, Nature, PNAS, Cytoskeleton.
Membership: American Society for Cell Biology (ASCB), Microscopy Society of America (MSA);
Conference Session Chairs: Tomography Session at the Microscopy & Microanalysis Conference, San Antonio, TX (2003); "Isolated Proteins & Filaments" at the GRC on 3D Electron Microscopy, New London, NH (2007); Tomography Session at the Microscopy & Microanalysis Conference, Ft. Lauderdale, Florida (2007); Mini-symposium on "Molecular Motors: Alone and in Groups" at the 47th Annual Meeting of the ASCB (2007); Tomography session at the GRC on 3D Electron Microscopy, Lucca, Italy (2008); Chair of Tomography Session at the Microscopy & Microanalysis Conference, Phoenix, AZ (2012); Session chair at the 7th International Electron Tomography Conference, Cancun, Mexico (2014).
Instructional activities: IMOD workshop, Boulder, CO (2003, 2004); Cryo-EM Workshops at the Scripps Research Institute, La Jolla, CA (2003); Guest lecturer at Cold-Spring Harbor Laboratories (2006); Workshop Speaker at the CBS/Imaging Center and MRSEC at the University of Minnesota, MN (2008); EMBO electron tomography workshop in Leiden, Netherlands (2009); Workshop on 3D Solutions in Cryo-Electron Microscopy in Barcelona, Spain (2013).

Honors

1996-1998	Graduate scholarship for Junior Scientists by Ludwig-Maximilians-University & Bavaria, Germany.
1998-2000	Graduate scholarship from the Max-Planck-Gesellschaft, Germany.
2000-2001	Postdoctoral scholarship from the Max-Planck-Gesellschaft, Germany.
2007-2011	Pew Scholar in the Biomedical Sciences
2008	Strage Award for Aspiring Young Science Faculty
2008	WM Keck Foundation Research Excellence Award
2008-	LAS (Local Affiliated Societies) tour speaker for the Microscopy Society of America
2009-2012	WM Keck Foundation Science & Engineering Research Grants Award
2014	Keith R. Porter Fellow

C. Contribution to Science

1. Cilia and flagella are the most complex microtubule assemblies, and although they have been studied intensely for more than a century, many open questions remain. For the past ten years, we have taking a systems approach and applied both bottom-up and top-down strategies to study the composition, structure and function of cilia, which has both driven methods development (see point 4 below) and resulted in a renaissance of the field, with discoveries continuing to mount. We use a unique combination of approaches, integrating high-resolution in-situ-imaging and image processing (cryo-electron tomography, subtomogram averaging and TYGRESS), with genetics (WT-mutant comparisons, clonable tags) and biochemistry/proteomics (2D-PAGE and mass-spectrometry) to generate a molecular blueprint of these remarkable and biomedically important nano-machines. In collaboration with many experts in the cilia field, my group has now characterized the cilia of 6 evolutionarily distant species – from algae to human – comparing their WT structure with ~40 mutants, including a first translational study of cilia from a human ciliopathy patient. We have dissected the structures of 9 major ciliary complexes at ~3 nm resolution and discovered novel structures and their 3D organization, such as the N-DRC (nexin-dynein regulatory complex), OID (outer-inner-dynein-linker), a novel protein family called MIPs (Microtubule Inner Proteins), the I1-dynein-tether that connects a dynein-head to the microtubule, doublet-specific-features, the MIA-complex that is required for stable assembly of I1-dynein, radial spoke heterogeneity and the CSC (CaM- and spoke-associated-complex), resulting in already 20 research articles.

    a) Nicastro D, Schwartz C, Pierson J, Gaudette R, Porter M, McIntosh JR (2006) The molecular architecture of axonemes revealed by cryoelectron tomography. Science 313:944-8.
    b) Heuser T, Raytchev M, Krell J, Porter ME, Nicastro D (2009) The dynein regulatory complex is the nexin link and a major regulatory node in cilia and flagella. J Cell Biol. 187:921–933.
    c) Nicastro D, Fu X, Heuser T, Tso A, Porter ME, Linck R (2011) Cryo-electron tomography reveals conserved features of doublet microtubules in flagella. PNAS USA 108:E845-853.
    d) Heuser T, Barber CF, Lin J, Krell J, Rebesco M, Porter ME, Nicastro D (2012) Cryoelectron tomography reveals doublet-specific structures and unique interactions in the I1 dynein. PNAS USA 109:E2067-76.

e) Lin J, Yin W, Smith MC, Song K, Leigh MW, Zariwala MA, Knowles MR, Ostrowski, Nicastro D (2014) Cryo-electron tomography reveals ciliary defects underlying human RSPH1 primary ciliary dyskinesia. Nature Commun. 5:5727.

2. Dynein is the largest and most elusive of the cytoskeletal motors. Despite of recent crystal structures of the dynein motor domain in the post-powerstroke ("inactive") state, little was known about the high-resolution 3D structure of the pre-powerstroke ("active") conformation, and thus of the mechanism of dynein motility. My lab analyzed cryo-tomograms of intact sea urchin sperm flagella that were actively beating while they were rapidly frozen. Using classification tools to separate different conformational states before generating class averages, we were able to determine the *in situ* 3D structures of both pre- and post-powerstroke dynein, including two distinct pre-powerstroke conformations: pre-I (microtubule-detached) and pre-II (microtubule-bound). The three conformations provided direct evidence that dynein undergoes a head-swing rather than a linker-swing mechanism inside cells, and allowed us to propose a molecular model for the structural cycle underlying dynein movement. We are in the process of analyzing conformational changes of all major complexes in actively beating cilia, which suggests a new robust mechanism for ciliary and flagellar motility by targeted dynein inhibition rather than activation, and provides better understanding of the distinct roles played by various dyneins and regulators in the motility of cilia.

    a) Lin J, Okada K, Raytchev M, Smith MC, Nicastro D (2014) Structural mechanism of the dynein powerstroke. Nature Cell Biol. 16:479–485.

3. Although my lab's expertise is mainly cellular cryo-electron tomography to achieve molecular resolution, I'm also a classically trained structural biologist and see the continued need for more traditional yet modernized electron microscopy studies on a cellular, tissue and organism level using e.g. high-pressure freezing/freeze substitution preparation, correlative light and electron microscopy (CLEM) and three-dimensional EM (i.e., serial section TEM and plastic section electron tomography). Therefore I built and directed the Brandeis Facility for Correlative Light and Electron Microscopy, through which I interacted with biologists, biochemists, biophysicists, and chemists to help with their research projects, ranging from studying neurons in a round worm, actin nucleation in heart muscle cells, membrane deformation by FBAR and viral proteins, to molecular crowding of bacteriophages, artificial cilia and other bio-inspired, engineered materials. For example, I realized my vision of a traditional structural atlas of the anterior nervous system in *C. elegans* using modern EM techniques, resulting in a 35-page/29-figures eLife paper and reference for future studies; these and supplementary data will also be made available on the wormatlas.org.

    a) Gopalakrishnan J, Mennella V, Blachon S, Zhai B, Smith A, Megraw T, Nicastro D, Gygi S, Agard D, Avidor-Reiss T (2011) Sas 4 scaffolds cytoplasmic complexes and tethers them in a centrosome. Nature Commun 2:359, DOI: 10.1038/ncomms1367.
    b) Gibaud T, Barry E, Zakhary MJ, Henglin M, Ward A, Yang Y, Berciu C, Oldenbourg R, Hagan MF, Nicastro D, Meyer RB, Dogic Z (2012) Reconfigurable self-assembly through chiral control of interfacial tension. Nature 481: 348-51.
    c) Rosado M, Barber CF, Berciu C, Feldman S, Birren S, Nicastro D, Goode BL (2014) Critical roles for multiple formins during cardiac myofibril development and repair. Mol Biol Cell 25:811-27.
    d) Doroquez DB*, Berciu C*, Anderson JR, Sengupta P, Nicastro D (2014) A high-resolution morphological and ultrastructural map of anterior sensory cilia and glia in *C. elegans*. eLife 3:e01948.
    e) Eisele DM, Arias DH, Fu X, Bloesma EA, Steiner CP, Jensen RA, Rebentrost P, Eisele H, Tokmakoff A, Lloyd S, Nelson KA, Nicastro D, Knoester J, Bawendi MG (2014) Robust excitons inhabit soft supramolecular nanotubes. PNAS USA 111:E3367-75.
    f) Kelley CF, Messelaar EM, Eskin TL, Wang S, Song K, Vishnia K, Becalska AN, Shupliakov O, Hagan MF, Danino D, Sokolova OS, Nicastro D, Rodal AA (2015) Membrane Charge Directs the Outcome of F-BAR Domain Lipid Binding and Autoregulation. Cell Rep. 13:2597-609. [PMCID: in progress]

4. Driven by important biological questions, I have developed cellular imaging techniques and methods that allow studying cellular and macromolecular structures *in situ* and with as high as possible resolution. As postdoctoral fellow, I introduced cryo-electron tomography to the Boulder Lab for 3D EM of Cells and then

initiated the development of hardware (cryo-light microscopy stage for cryo-CLEM, cryo-focused ion beam for frozen-hydrated sectioning) and software for subtomogram averaging (PEET: particle estimation for electron tomography). As PI I have continued to push boundaries to improve the resolution and/or information content of cellular imaging, with the long-term goal to realize the full potential of EM and cryo-ET as general techniques to routinely yield high-resolution reconstructions of cellular structures. To overcome current resolution and contrast limitations of cellular cryo-ET, we designed a new approach called Tomography-Guided 3D Reconstruction of Subcellular Structures (TYGRESS, soon available at tygress.org), which has improved the resolution of the axonemal repeat in 250 nm thick, intact cilia from ~3 nm to ~1.5 nm resolution. We have also advanced clonable EM-visible protein labels and methods for CLEM. We envision that in the future cellular cryo-ET will smoothly link light microscopy of living cells and x-ray crystallography of isolated macromolecules, allowing atomic models to be docked directly into tomographic averages, and thus providing atomic maps of cells in healthy and diseased states.

a) Nicastro D, Schwartz C, Pierson J, Gaudette R, Porter M, McIntosh JR (2006) The molecular architecture of axonemes revealed by cryoelectron tomography. Science 313:944-8.
b) Basgall EJ and Nicastro D (2006) Cryo-Focused Ion Beam Preparation of Biological Materials for Retrieval and Examination by Cryo-TEM. Microscopy and Microanalysis 12(Supplement S02):1132-1133.
c) Schwartz CL, Sarbash VI, Ataullakhanov FI, McIntosh JR, Nicastro D (2007) Cryo-fluorescence microscopy facilitates correlations between light and cryo-electron microscopy and reduces the rate of photobleaching. J Microsc. 227: 98-109.
d) Song K, Awata J, Tritschler D, Bower R, Witman GB, Porter ME, Nicastro D (2015) In situ localization of N- and C-termini of subunits of the flagellar nexin-dynein regulatory complex (N-DRC) using SNAP-tag and cryo-electron tomography. J Biol Chem. 290:5341-53.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/daniela.nicastro.1/bibliography/43833951/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

R01 GM083122 Nicastro (PI) 10/01/07-06/30/16
NIH
"Determining the Structure, Function and Regulation of Dynein and Flagella"
The major goals of this project are to study the structure and function of the molecular motor dynein using both purified cytoplasmic dynein bound to microtubules, and axonemal dynein from *Chlamydomonas* and sea urchin sperm flagella. We are also studying dynein regulation by identifying the organization of the I1 inner dynein complex and the dynein regulatory complex.
Role: PI

1-FY13-478 Nicastro (PI) 07/01/13-06/30/16
March of Dimes
"Structure and Function of the Central Pair Complex in Cilia and Flagella"

The major goal of this project is a better understanding of the three-dimensional structure, subunit composition, protein interactions and regulatory functions of the central pair complex in cilia and flagella.
Role: PI

1158006 Fraden, Dogic, Nicastro (PIs) 11/01/13-06/30/16
NSF
"Hierarchical assembly and dynamics of cilia-like structures"
Continuation of projects started under the completed Keck Award, with the specific aims being: 1) Molecular motor induced microtubule sliding *in vitro*; 2) Transforming of *in vitro* microtubule sliding into bending; 3) Beating of multifilament bundles and synchronization of dense bundle and fields of cilia.
Role: Co-PI

R01 GM111506 Nicastro (PI) 09/01/14-08/31/18
NIH
“Resolution Improvement for Cellular CryoEM to study Dynamic Assemblies in Cells”
The major goals of this project are to develop a new hybrid approach and software tool, called TYGRESS, to improve the resolution of cellular cryo-electron microscopy to a level at which protein domains can be recognized and compared to known atomic structures. We will build the infrastructure to make this new tool available to a wide scientific community. We will also apply this new tool to study the structure and function of three important and dynamic assemblies inside cells, i.e. the molecular organization and function of cilia and flagella, rotavirus entry and host cell infection, and the protein transport machinery across or into the ER membrane.
Role: PI

CPRIT rising star recruitment award Nicastro (PI) 09/01/14-08/31/18
Cancer Prevention Research Institute of Texas
“High-resolution visualization of native, dynamic DNA-interacting complexes inside normal and cancerous cells”
The major goals of this project are an *in situ* structure-function study of several major, cancer-relevant, DNA-interacting assemblies. We will generate 3D reconstructions with molecular resolution and 4D movies (i.e., time-resolved sequences of 3D structures) of the highly dynamic DNA damage response complexes, of telomerase, as it cycles between Cajal bodies and telomeres, and of the elusive promyelocytic leukemia nuclear bodies. Each of these complexes/assemblies will be studied in several cell types and phases of the cell cycle, providing a detailed comparison of their structures in normal and cancerous cells.
Role: PI

Completed Research Support (last three years)

Keck Award Fraden, Dogic, Nicastro (PIs) 01/01/10-12/31/14
WM Keck Foundation/ Science & Engineering Research
"Active matter at micro, meso and macroscopic scales"
The goal of this proposal is to elucidate the physical behavior of active matter, i.e. agents that consume energy to generate motion, using an interdisciplinary approach involving both physics and biology. We use a) a "bottom up" approach to determine the minimal system required to create a self-oscillating active microtubule bundle *in vitro*; b) a complementary "top down" approach to deconstruct a fully functional axoneme and determine the minimal set of structural components required for its active beating using high-speed video imaging and laser trap microscopy; and c) active nematic liquid crystals will be assembled and characterized.
Role: Co-PI